Natural stilbenoids have anti-inflammatory properties in vivo and down-regulate the production of inflammatory mediators NO, IL6 and MCP1 possibly in a PI3K/Akt-dependent manner

Journal:	*Journal of Natural Products*
Manuscript ID	np-2017-00384z.R3
Manuscript Type:	Full Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Eräsalo, Heikki; University of Tampere, Faculty of Medicine and Life Sciences, Pharmacology Hämäläinen, Mari; University of Tampere, Faculty of Medicine and Life Sciences Leppänen, Tiina; University of Tampere, Faculty of Medicine and Life Sciences, Pharmacology Mäki-Opas, Ilari; University of Tampere, Faculty of Medicine and Life Sciences, Pharmacology Laavola, Mirka; University of Tampere, Faculty of Medicine and Life Sciences, Pharmacology Haavikko, Raisa; University of Helsinki, Department of Chemistry Yli-Kauhaluoma, Jari; University of Helsinki, Faculty of Pharmacy Moilanen, Eeva; University of Tampere, Faculty of Medicine and Life Sciences, Pharmacology

Natural stilbenoids have anti-inflammatory properties *in vivo* and down-regulate the production of inflammatory mediators NO, IL6 and MCP1 possibly in a PI3K/Akt-dependent manner

Heikki Eräsalo[†], Mari Hämäläinen[†], Tiina Leppänen[†], Ilari Mäki-Opas[†], Mirka Laavola[†], Raisa Haavikko[‡], Jari Yli-Kauhaluoma[‡], Eeva Moilanen[†*]

[†]The Immunopharmacology Research Group, Faculty of Medicine and Life Sciences, University of Tampere and Tampere University Hospital, FI-33014 Tampere, Finland

[‡]Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, FI-00014 Helsinki, Finland

ABSTRACT

Stilbenoids are a group of polyphenolic compounds found in plants, trees, berries and nuts. Stilbenoids have been shown to serve an anti-microbial and anti-fungal function in plants. There is also evidence that as a part of human diet, stilbenoids play an important role as antioxidants and may have anti-inflammatory effects. The PI3K/Akt pathway is a well-characterized signaling pathway controlling cellular functions involved in growth and cell cycle, and in metabolism. There is also increasing evidence to show the involvement of this pathway in the regulation of inflammatory responses. In the present study, an attempt was made to investigate the anti-inflammatory properties of naturally occurring stilbenoids pinosylvin (**1**), monomethyl pinosylvin (**2**), resveratrol (**3**), pterostilbene (**4**), piceatannol (**5**) and rhapontigenin (**6**). Glycosylated derivatives of piceatannol and rhapontigenin, namely astringin (**7**) and rhaponticin (**8**), respectively, were also investigated. In addition to the natural stilbenoids, pinosylvin derivatives (**9-13**) were synthesized and subjected to the testing of their effects on PI3K/Akt pathway in inflammatory conditions.

The investigated natural stilbenoids (except the glycosylated derivatives) were found to down-regulate Akt phosphorylation, which is a well acknowledged marker for PI3K activity. It was also found that all of the studied natural stilbenoids had anti-inflammatory effects *in vitro*. The three most potent stilbenoids, piceatannol, pinosylvin and pterostilbene were selected for *in vivo* testing and were found to suppress the inflammatory edema and to down-regulate the production of inflammatory mediators IL6 and MCP1 in carrageenan-induced paw inflammation in the mouse. When compared to the commercial PI3K inhibitor LY294002, the anti-inflammatory effects appeared to be quite similar. The results reveal hitherto unknown anti-inflammatory effects of natural stilbenoids and suggest that those effects may be mediated via inhibition of the PI3K/Akt pathway.

Introduction

Stilbenoids are a group of polyphenolic compounds found in nature and particularly in plants. Stilbenoids have been shown to have anti-microbial and anti-fungal properties and it is likely that their role in plants is to serve as defensive compounds against microbial pathogens.[1] They are synthesized in trees as secondary metabolites during heartwood biogenesis and they are also actively produced as a response to infection or injury. Stilbenoids can therefore be also classified as phytoalexins due to their antimicrobial properties.[2,3]

Due to being abundant in plants, stilbenoids are also present in human nutrition. Their health effects and nutritional significance has also been studied. Resveratrol, probably the most well-known and investigated stilbenoid, is present in grapes, different berries, nuts and also in red wine.[4] Resveratrol has been reported to have anti-inflammatory, anti-carcinogenic, anti-oxidative and cardioprotective properties.[5] However, in addition to resveratrol, several other stilbenoid compounds are present in human nutrition. For instance, pterostilbenoid, rhapontigenin and piceatannol can all be found in different berries, fruits, nuts and other edible plants.[6-10] Structurally very closely related stilbenoids, pinosylvin and monomethyl pinosylvin, are also readily found in pine knots[11] and also in pine nuts.[12] There are fragmentary data on the health promoting effects of those stilbenoids, sometimes resembling the effects of resveratrol[13] including some anti-inflammatory effects.[14-16]

The PI3K/Akt pathway is a cellular signaling pathway involved in various cellular processes, such as cell proliferation, migration and survival as well as glucose metabolism. It is also considered to have a remarkable role in inflammatory responses[17] and its role in cancer is also vastly studied.[18] The PI3K/Akt pathway consists of phosphatidylinositol 3-kinases that catalyze the phosphorylation of phosphatidylinositols, a class of structurally important lipid molecules in cell membranes. The phosphatidylinositolphosphates (PIPs) generated in the

process then serve as a docking platform for Akt kinase (also known as PKB). Akt kinase is a serine/threonine kinase that has a wide range of downstream targets regulating different processes, such as cell proliferation, glucose metabolism, neuronal development as well as inflammation.[19-21] It has been shown that when the PI3K/Akt pathway is inhibited with pharmacological compounds, the cellular inflammatory responses are attenuated due to down-regulation of inflammatory genes.[22-24]

The aim of the present study was to investigate natural stilbenoids (Figure 1), including: pinosylvin (**1**), monomethyl pinosylvin (**2**), resveratrol (**3**), pterostilbene (**4**), piceatannol (**5**) and rhapontigenin (**6**) and test their effects on the PI3K/Akt pathway in activated macrophages. Additionally, the glycoside derivatives of piceatannol and rhapontigenin, namely, astringin (**7**) and rhaponticin (**8**), respectively, were investigated. In addition to the natural stilbenoids, pinosylvin derivatives (**9-13**) were synthesized and subjected to the anti-inflammatory testing. Another aim was to investigate the effects of the aforementioned stilbenoids on the production of the inflammatory mediators interleukin 6 (IL6), monocyte chemotactic protein 1 (MCP1) and nitric oxide (NO). Further, the anti-inflammatory effects of three potent stilbenoids (piceatannol, pinosylvin and pterostibene) were also investigated *in vivo* in the carrageenan-induced paw inflammation model in the mouse. Additionally the *in vivo* studies were backed up with *ex vivo* studies by measuring inflammatory cytokines in the inflamed paw tissue. The results reveal that all of the investigated natural stilbenoids (excluding the glycoside derivatives) have an inhibitory effect on the activation of the PI3K/Akt pathway. It was also found that these compounds down-regulate the production of inflammatory mediators, possibly via a PI3K/Akt dependent mechanism. An anti-inflammatory effect was also observed in the *in vivo* model and was further elucidated with *ex vivo* studies.

RESULTS

Synthesis of pinosylvin derivatives

A panel of pinosylvin derivatives bearing different alkyloxy and allyloxy moieties (compounds **9-13**) was designed (Figure 1). The compounds were synthesized by a nucleophilic substitution reaction between the commercially available pinosylvin and different alkyl (Et, *n*-Pr, Bn, and *i*-Bu) and allyl bromide in the presence of potassium carbonate as a base in acetone. The novel pinosylvin ethers were produced in low to moderate yields.[25] Their chemical structures were confirmed by ^{1}H and ^{13}C NMR spectroscopy.

Natural stilbenoids inhibited PI3K/Akt activation in a concentration-dependent manner in J774 macrophages

Stimulation of macrophages through a TLR4-mediated mechanism by exposing the cells to bacterial endotoxin LPS (10 ng/mL) induced PI3K-mediated phosphorylation of Akt protein on amino acid residue S473 and treating the cells with the commercial PI3K inhibitor LY294002 inhibited Akt phosphorylation (Figure 2). Next, the effects of natural stilbenoids pinosylvin (**1**), monomethyl pinosylvin (**2**), resveratrol (**3**), pterostilbene (**4**), piceatannol (**5**) and rhapontigenin (**6**) on the phosphorylation of Akt in activated macrophages were investigated. The results showed that all of the investigated stilbenoids inhibited Akt phosphorylation in a concentration-dependent manner (Fig 3 A-F). Piceatannol (5) was the most potent of the tested stilbenoids followed by monomethyl pinosylvin (2) and pinosylvin (1) which all inhibited Akt phosphorylation in a statistically significant manner already at 3 μM concentrations while rhapontigenin (6) appeared to be the least potent in the series of the natural stilbenoids. The glycoside derivatives of piceatannol and rhapontigenin, i.e., astringin (**7**) and rhaponticin (**8**), respectively, were also investigated. The glycosides had no effect on Akt phosphorylation (Fig 3).

Semi-synthetic pinosylvin derivatives exhibited a structure-related loss of effect on Akt phosphorylation when compared to natural stilbenoids

In order to compare the effects of natural stilbenoids versus synthetic pinosylvin derivatives, Akt phosphorylation was stimulated with LPS in J774 macrophages treated with the pinosylvin derivatives **9**-**13**. Pinosylvin (**1**) was used as a reference compound in the experiments. It was found that although some of the synthetic stilbenoid derivatives inhibited Akt phosphorylation the effect was not comparable in magnitude to the effect of the natural stilbenoid pinosylvin (Fig 4): Monoethyl pinosylvin (**9**) and monoallyl pinosylvin (**11**) reduced Akt phosphorylation in a statistically significant manner when used at 30 μM concentrations. Monoisobutyl pinosylvin (**12**) and monobenzyl pinosylvin (**13**) had no effect at 30 μM concentrations while monopropyl pinosylvin (**10**) appeared to have a small inhibitory effect but it did not reach statistical significance. The loss of effect appeared to correlate with the length of the carbon side chain on the hydroxy group connected to the R1 position in the phenyl ring.

Natural stilbenoids down-regulated the production of inflammatory mediators NO, IL6 and MCP1 in J774 macrophages

In order to investigate the effects of the natural stilbenoids **1**-**8** on the production of inflammatory mediators NO, IL6 and MCP1 in activated macrophages, the J774 macrophage cells were stimulated with LPS in the presence of increasing concentrations of the stilbenoids for 24 hours prior to cell culture media collection. It was found that the investigated stilbenoids had a concentration-dependent inhibitory effect on the production of the aforementioned inflammatory mediators, and that was comparable to the effects obtained with the known PI3K inhibitor LY294002. (Figures 5-7)

The control compound LY294002 inhibited NO production by 50% at 8.9 μM concentration. Closely comparable values were obtained for resveratrol (**3**, 12.15 μM), pterostilbene (**4**, 16.7 μM) and pinosylvin (**1**, 16.6 μM). Clearly higher values were obtained for monomethyl pinosylvin (**2**, 37.0 μM) and rhapontigenin (**6**, 102.8 μM). For piceatannol (**5**) the value was the lowest (2.6 μM). (Figure 5)

LY294002 inhibited IL6 expression half-maximally at 5.1 μM concentration. Comparable to this were resveratrol (**3**, 6.8 μM), pterostilbene (**4**, 8.7 μM) and piceatannol (**5**, 13.0 μM). Higher values were detected with pinosylvin (**1**, 32.1 μM), monomethyl pinosylvin (**2**, 30.2 μM) and rhapontigenin (**6**, 99.1 μM). (Figure 6)

For MCP1 production, LY294002 had a 50% inhibitory effect at 3.7 μM. The only stilbenoid comparable to that was piceatannol (**5**, 4.0 μM) and perhaps pterostilbene (**4**, 10.5 μM). Clearly higher values were found for pinosylvin (**1**, 38.7 μM), monomethyl pinosylvin (**2**, 23.7 μM), resveratrol (**3**, 13.6 μM) and rhapontigenin (**6**, 19.4 μM). (Figure 7)

In contrast, the glycoside derivatives of piceatannol and rhapontigenin, i.e., astringin (**7**) and rhaponticin (**8**), respectively, appeared to have lost their activity when compared to the corresponding non-glycosylated stilbenoid (Figures 5-7).

Natural stilbenoids showed anti-inflammatory effects both *in vivo* and *ex vivo*

Carrageenan-induced paw edema mouse model was used as an *in vivo* model of inflammation to investigate the anti-inflammatory effects of natural stilbenoids. Pinosylvin (**1**), pterostilbene (**4**) and piceatannol (**5**) were selected for the *in vivo* studies due to them having similar effects and relatively similar inhibition percentages in the *in vitro* studies than the PI3K inhibitor LY294002. Also, based on the literature search pterostilbene (**4**) and piceatannol (**5**) appeared also to be the least investigated stilbenoids with very little published

data from *in vivo* experiments. A widely used anti-inflammatory drug dexamethasone was used as the control compound. It was found that all of the investigated natural stilbenoids, i.e., **1**, **4** and **5** (when given at the dose of 30 mg/kg), had an anti-inflammatory effect *in vivo* comparable to that of LY294002 (15 mg/kg) and dexamethasone (2 mg/kg), (Figure 8).

In addition to the *in vivo* studies, *ex vivo* studies were conducted by measuring the levels of inflammatory cytokines IL6 and MCP1 in the mouse paw tissue after carrageenan injection. It was found that the inflamed paw tissue from mice treated with either stilbenoids or the control compounds LY294002 or dexamethasone showed a diminished inflammatory response, which was seen as lower expression of the inflammatory cytokines IL6 and MCP1 (Figure 9).

DISCUSSION

In the present study, our aim was to investigate anti-inflammatory properties of natural stilbenoids pinosylvin (**1**), monomethyl pinosylvin (**2**), resveratrol (**3**), pterostilbene (**4**), piceatannol (**5**), rhapontigenin (**6**), astringin (**7**) and rhaponticin (**8**).The hypothesis was that stilbenoids would down-regulate inflammatory gene expression and the activity of the PI3K/Akt pathway in relation to inflammation. The results obtained in this study support the idea that natural stilbenoids have anti-inflammatory properties and the effect is possibly mediated via inhibition of the PI3K/Akt pathway. Further, it was found that the investigated semi-synthetic pinosylvin derivatives showed weaker effects on PI3K/Akt pathway in inflammatory conditions when compared to their naturally occurring counterparts.

The health-beneficial properties of the stilbenoid resveratrol and red wine have been a popular research topic ever since the concept of the French paradox was formulated in the 1980s.[26] Reports have shown that resveratrol has anti-inflammatory, anti-carcinogenic, anti-oxidative and cardioprotective properties[5] as well as beneficial effects on aging, diabetes and neurological dysfunction.[27] The *in vitro* anti-inflammatory effects of resveratrol were also reproduced in the present study. However, there exists a plethora of foodstuffs containing stilbenoids other than resveratrol, such as different berries, nuts and other edible plants which are a natural part of human nutrition worldwide. Therefore, in this study we focused mainly on other stilbenoids besides resveratrol, namely pinosylvin (**1**), monomethyl pinosylvin (**2**), pterostilbene (**4**) piceatannol (**5**), and rhapontigenin (**6**) as well as five synthetic derivatives of pinosylvin.

The PI3K/Akt signaling pathway controls an array of cellular functions, some of which are also involved in inflammation. Regarding inflammation, the PI3K/Akt pathway is documented to regulate survival, motility, differentiation and proliferation of leukocytes via

cytokine and chemokine signaling as well as NF-κB mediated transcription.[21] Among three different classes of PI3Ks there also exists several different PI3K isoforms. The class I PI3Ks are considered to be involved in inflammation and particularly class I PI3K subunits PI3Kδ and PI3Kγ which are considered to regulate cytokine signaling and chemotaxis of immune cells.[22,28]

Low-grade chronic inflammation has been documented to be increasingly prevalent especially in western countries. Western lifestyle and diet has been suggested as one of the main causes of low-grade inflammation and the prevalence of metabolic syndrome and type 2 diabetes is considered to be a direct effect caused by low-grade inflammation.[29] Obesity is also known to up-regulate inflammation and it has been reported that macrophages located in the adipose tissue produce significant amounts of IL6 and MCP1 as well as other inflammatory mediators.[30] It has also been documented that a healthy diet containing phytonutrients from fruits and vegetables modulates low-grade inflammation towards a more healthy level.[31] A recent study also documented that the PI3K/Akt pathway is activated in low-grade inflammation and that the inflammation can be attenuated with PI3K inhibition.[32] Based on our present findings we hypothesize that a diet containing natural stilbenoids could possibly also attenuate low-grade inflammation via a PI3K-dependent mechanism.

In this study we used a commercially available PI3K inhibitor LY294002 as a reference compound for PI3K inhibition. LY294002 is a well-characterized class I PI3K inhibitor.[33] The phosphorylation of Akt is often considered as a marker for PI3K activity. Upon binding the phosphatidylinositols produced by PI3Ks, Akt is docked to the cell membrane, which allows PDK1 and MTOR to phosphorylate it.[20] Our results show that the control compound PI3K inhibitor LY294002 used in this study, inhibits Akt phosphorylation upon LPS stimulation also in activated macrophages and inhibits the production of inflammatory cytokines IL6 and MCP1 *in vitro* and *in vivo* as expected.

Based on our findings, it appears that the investigated stilbenoids also exhibit similar inhibitory effects as the PI3K inhibitor LY294002 on the phosphorylation of Akt indicative of the inhibition of the activity of the PI3K/Akt pathway. These data are supported by the few studies that have focused on the effects of stilbenoids on the PI3K/Akt pathway in other cell types and experimental settings.[14,34,35] However, the findings reported in this study suggest that among the investigated stilbenoids there are differences regarding the PI3K/Akt inhibition potency. Piceatannol (compound **5**) appeared to be the most potent of the tested natural stilbenoids, followed by pinosylvin (compound **1**) and monomethyl pinosylvin (compound **2**) while rhapontigenin (compound **6**) was the least potent. Very similar pattern of activity was also seen in their inhibitory effects on the production of the inflammatory factors nitric oxide, IL6 and MCP1, see below.

The semi-synthetic pinosylvin derivatives were less potent than the natural stilbenoid pinosylvin, and the loss of activity seemed to associate with the length of the side chain on the R1 position in the left-hand side phenyl ring. When the pinosylvin derivatives were compared to the natural pinosylvin, it appeared that a small substituent such as methyl on the R1 carbon did not significantly effect the potency of PI3K/Akt inhibition as was documented with monomethyl pinosylvin (compound **2)**. However, when the substituent on R1 became longer, such as the ethyl, propyl or isobutyl ether group on compounds **9**, **10** and **12**, respectively, or bulkier such as the allyl or benzyl ether groups on compounds **11** and **13**, respectively, the effect observed on PI3K/Akt inhibtion was clearly weakened. In fact, compounds 12 and 13 had no effect at 30 μM concentrations, and compound 10 had a minor effect which did not reach statistical significance. This is possibly due to the fact that longer or bulkier substituents are more likely to cause steric hindrance in the interaction mechanisms between these molecules.

In addition, the glycosidic derivatives of piceatannol and rhapontigenin, namely astringin and rhaponticin, respectively, did not appear to have an effect on the PI3K/Akt pathway. This is possibly due to the same mechanism described above and / or related to the fact that glycoside derivatives are not readily transported into the cells but rather stay in the cell culture medium where they remain inactive. To further elucidate this, it would be meaningful to investigate other different glycosidic derivatives (eg. glycosides with galactose or fructose conjugates) because both of the tested compounds were O-beta-D-glycoside derivatives of the stilbenoids in question.

Nitric oxide produced by inducible nitric oxide synthase (iNOS) as well as the cytokines IL6 and MCP1 are well known inflammatory mediators. Piceatannol (compound **5**) was the most potent of the tested stilbenoids in decreasing nitric oxide production while rhapontigenin (compound **6**) was the least potent of the non-glycosylated stilbenoids; and the potency of the other non-glycosylated stilbenoids was between those two. The glycosylated stilbenoids astringin (compound **7**) and rhaponticin (compound **8**) did not have any effect. Very similar pattern of activity was also seen in their inhibitory effects on Akt phosphorylation, and IL6 and MCP1 production. Rhapontigenin (**6**) is the only aglycone which has a methyl group at position C4 while piceatannol (**5**) has hydroxy groups in positions R1, R3, R4 and R5 which may contribute to their anti-inflammatory activity. The glycosylated stilbenoids remained ineffective possibly because they are not readily transported into the cells which is necessary for their effects on the production of inflammatory mediators.

The production of IL6, MCP-1 and nitric oxide is activated by various inflammatory stimuli and also different transcription factors are documented to be involved. The cellular effects of PI3K are occasionally reported to be mediated via the NF-κB transcription factor and the three measured inflammatory mediators are also reported to be NF-κB responsive.[36-39] Our findings document that inhibition of PI3K/Akt pathway with LY294002 down-regulates the

expression of these inflammatory mediators, which in turn suggests that these inflammatory mediators are PI3K/Akt responsive. It was also found that the natural stilbenoid compounds investigated in the present study down-regulated the expression of these inflammatory mediators. When these data are considered alongside with our findings that the stilbenoids also inhibited PI3K/Akt activation, it is plausible that the down-regulated inflammatory response is mediated via PI3K inhibition.

Carrageenan-induced paw edema is a well-characterized *in vivo* model of acute inflammation. It has been widely reported that the inflammatory factors mediating carrageenan-induced edema include IL6, MCP1 and NO.[40-42] The PI3K/Akt pathway is also considered to be one of the signaling pathways involved in acute inflammation such as the inflammatory edema caused by exposure to carrageenan. We and others have earlier documented that inhibition of the PI3K/Akt pathway leads to reduced edema and decreased inflammatory cell migration upon carrageenan irritation.[21,24] These data support the idea that PI3K/Akt signaling is involved in mediating the formation of the inflammatory response induced by exposure to carrageenan. In the present study, we found that the investigated natural stilbenoids also reduced the carrageenan-induced edema and suppressed the expression of the inflammatory cytokines IL6 and MCP1 in the inflamed paw tissue. According to our knowledge, this is a novel *in vivo* effect for pterostilbene (**4**) and piceatannol (**5**) that has not been previously reported. The effect appears to be comparable to the effect obtained with the commercial PI3K inhibitor LY294002. This in turn suggests that the investigated stilbenoids would have anti-inflammatory properties *in vivo* and that the mechanism of the effect possibly includes an inhibitory interaction with the PI3K/Akt pathway.

In this study we document our findings regarding several naturally occurring stilbenoid compounds, i.e. pinosylvin, monomethyl pinosylvin, resveratrol, pterostilbene, piceatannol

and rhapontigenin, which are all known to be included in a normal healthy human diet. It was found that these stilbenoids have anti-inflammatory properties when investigated *in vitro* and *in vivo* along with their inhibitory action on the PI3K/Akt signaling pathway. The effects were comparable to the effects obtained with the known PI3K/Akt inhibitor LY294002. Additionally, we found that the tested glycoside derivatives of these stilbenoids did not have a significant effect in the inflammatory models. Based on these findings it appears that the naturally occurring stilbenoids included in human nutrition have anti-inflammatory properties and it also appears that these effects could possibly be mediated via the PI3K/Akt pathway. This may be a significant finding that could be exploited to alleviate inflammatory conditions by a stilbenoids-containing dietary intervention but further studies are needed to understand the mechanisms of action and to prove bioavailability and actual clinical efficacy in man.

EXPERIMENTAL SECTION

Materials

The reagents used in the study were obtained as following: pAkt S473 and Akt antibodies from Cell Signaling Technology, Danvers, MA, USA; β-actin antibodies and HRP-conjugated goat polyclonal anti-rabbit IgG antibodies from Santa Cruz Biotechnology, Santa Cruz, CA, USA; PI3K inhibitor LY294002 from Merck Millipore, Billerica, MA, USA; pinosylvin, monomethyl pinosylvin and resveratrol from Sequoia Research Products, Pangbourne, UK; pterostilbene, piceatannol and dexamethasone from Orion Pharma, Finland; rhapontigenin from Cayman Chemical, MI, USA; astringin from Polyphenols Laboratories, Norway, and rhaponticin from Sigma Aldrich, MO, USA.

Chemistry

General experimental details

Commercially available reagents were used without further purification: all solvents were anhydrous and HPLC grade, purchased from Sigma-Aldrich (St. Gallen, Switzerland). All reactions were performed in oven-dried glassware under an inert atmosphere of dry argon. Thin layer chromatography (TLC) was performed on E. Merck Silica Gel 60 aluminium packed plates, visualization accomplished by UV illumination and staining with H_2SO_4 (5% v/v) in MeOH. ^{1}H NMR spectra were recorded on a Varian Mercury-VX 300 MHz with chemical shifts reported as parts per million (in acetone-d_6 at 23 °C, solvent peak at 2.05 ppm as an internal standard). ^{13}C NMR spectra were obtained on a Varian Mercury-VX 75 MHz spectrometer with chemical shifts reported as parts per million (acetone-d_6 at 23 °C, solvent peaks at 29.84, and 206.26 ppm as an internal standard).

Synthesis of pinosylvin monoethers

General procedure: A mixture of pinosylvin (0.050 g, 0.24 mmol), potassium carbonate (0.050 g, 0.36 mmol), and alkyl or allyl bromide (0.36 mmol) in acetone (3-5 mL) was refluxed under argon for 24 hours. The reaction mixture was poured into water and extracted with diethyl ether. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude products were purified with silica column eluting with 10-30 % hexanes/EtOAc to give monoethers in 8-28 % yields. **Pinosylvin monoethyl ether (9):** Off-white crystals (10.7 mg, 29%). R_f = 0.76 (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, acetone-d_6) δ 8.32 (s, 1H), 7.58 (m, 2H), 7.27 (m, 1H), 7.36 (m, 2H), 7.15 (d, J = 4.5 Hz, 2H), 6.68 (m, 2H), 6.35 (t, J = 2.2 Hz, 1H), 4.04 (q, J = 7 Hz, 2H), 1.35 (t, J = 7 Hz, 3H). **Pinosylvin monopropyl ether (10):** Starting from 69 mg (0.33 mmol) of pinosylvin as well as using 44 μL (0.49 mmol, 1.5 equiv) of 1-bromopropane, and 0.22 g of K_2CO_3 (1.6 mmol): White crystals (9.8 mg, 12%). R_f = 0.70 (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, acetone-d_6) δ 8.31 (s, 1H), 7.59 (m, 2H), 7.36 (m, 2H), 7.25 (m, 1H), 7.15 (d, J = 4.2 Hz, 2H), 6.68 (m, 2H), 6.36 (t, J = 2.2 Hz, 1H), 3.95 (t, J = 6.5 Hz 2H), 1.78 (m, 2H), 1.03 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 161.0, 158.9, 139.7, 137.7, 129.0, 128.8, 128.8, 127.8, 127.7, 126.7, 106.3, 104.3, 101.7, 69.3, 22.6, 10.1. **Pinosylvin monoallyl ether (11):** White crystals (17 mg, 28%). R_f = 0.82 (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, acetone-d_6) δ 8.34 (s, 1H), 7.58 (m, 2H), 7.37 (m, 2H), 7.25 (m, 1H), 7.15 (d, J = 4.7 Hz, 2H), 6.70 (m, 2H), 6.39 (t, J = 2.2 Hz, 1H), 6.06 (m, 1H), 5.43 (dq, J = 17.3, 1.5 Hz, 1H), 5.25 (dq, J = 10.6, 1.5 Hz, 1H), 4.57 (dt, J = 5.2, 1.6 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 160.5, 158.9, 139.7, 137.7, 134.2, 129.0, 128.9, 128.8, 127.8, 126.7, 116.6, 106.6, 104.5, 102.0, 68.6. **Pinosylvin monoisobutyl ether (12):** White crystals (4.8 mg, 7.5%). R_f = 0.77 (*n*-

hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, acetone-d_6) δ 8.29 (s, 1H), 7.58 (m, 2H), 7.37 (m, 2H), 7.25 (m, 1H), 7.15 (d, J = 4.5 Hz, 2H), 6.69 (m, 2H), 6.37 (t, J = 2.2 Hz, 1H), 3.77 (d, J = 6.5 Hz, 2H), 2.06 (m, 1H), 1.03 (d, J = 6.5 Hz, 6H); ^{13}C NMR (75 MHz, acetone-d_6) δ 161.1, 158.9, 139.7, 137.7, 129.0, 128.8, 128.8, 127.7, 126.7, 106.3, 104.4, 101.8, 74.3, 18.8.

Pinosylvin monobenzyl ether (13): White crystals (13 mg, 18%). R_f = 0.75 (*n*-hexane/EtOAc = 1:1). ^{1}H NMR (300 MHz, acetone-d_6) δ 8.37 (s, 1H), 7.62 – 7.54 (m, 2H), 7.53 – 7.47 (m, 2H), 7.45 – 7.31 (m, 5H), 7.30 – 7.19 (m, 1H), 7.16 (d, J = 4.7 Hz, 2H), 6.82 (dd, J = 7.2, 5.5 Hz, 1H), 6.76 – 6.65 (m, 1H), 6.45 (t, J = 2.2 Hz, 1H), 5.22 – 5.03 (m, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 161.3, 159.6, 144.6, 140.5, 138.5, 138.3, 132.1, 129.6, 129.5, 129.3, 128.6, 128.4, 127.4, 124.8, 107.4, 105.3, 102.8, 70.5.

Anti-inflammatory testing

Cell culture

Murine J774 macrophages (American Type Culture Collection, Manassas, VA, USA) were cultured at 37 °C in 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium with Ultraglutamine 1 (Sigma-Aldrich, MO, USA) supplemented with 10% heat-inactivated foetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and 250 ng/mL amphotericin B (all from Invitrogen, Paisley, UK). Cells were cultured on 24-well plates for western blot, ELISA, and nitrite measurements and on 96-well plates for cytotoxicity testing. Cells were grown for 72 h to confluence prior to the experiments.

Cytotoxicity of tested compounds was ruled out by measuring cell viability using Cell Proliferation Kit II (Roche Diagnostics, Mannheim, Germany).

Nitrite assays

The effects of the tested compounds on cellular NO production was investigated by measuring the accumulation of nitrite (NO_2^-, a stable metabolite of NO), in the cell culture medium with the Griess test[43].

IL6 and MCP1 ELISA

IL6 and MCP1 concentrations in the cell culture medium and paw tissue extracts were measured by enzyme linked immunosorbent assay (ELISA) using reagents from R&D Systems Europe Ltd (Abingdon, UK).

Western blotting

At indicated time points, cells were rapidly washed with ice-cold phosphate-buffered saline (PBS, pH 7.4) and lysed with a buffer containing 10 mM Tris-base (pH 7.4), 5 mM EDTA, 50 mM NaCl, 1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate, 20 μg/mL leupeptin, 50 μg/mL aprotinin, 5 mM NaF, 2 mM sodium pyrophosphate and 10 μM *n*-octyl-β-D-glucopyranoside. After incubation for 15 min on ice, cell lysates were centrifuged (13 000g, 4 °C, 10 min), supernatants were collected and stored in -20 °C in SDS sample buffer containing 0.5 M Tris (pH 6.8), glycerol (10% v/v), SDS (2% v/v), bromophenol blue (0.25‰ v/v) and 2-mercaptoethanol (5% v/v). An aliquot of the supernatant was used to determine protein concentration with the Bradford protein assay[44].

Protein samples (20 μg of total protein from lysates) were analysed according to standard western blotting protocol as described previously.[45] The membrane was incubated with the primary antibody in the blocking solution at 4 °C overnight, and with the secondary antibody in the blocking solution for 1 h at room temperature. Bound antibody was detected using Super Signal® West Pico or Dura chemiluminescent substrate (Pierce, Rockford, USA) and

Image Quant LAS 4000 imaging system (GE Healthcare Bio-Sciences, Buckinghamshire UK). The quantitation of the chemiluminescent signal was carried out with the use of Image Quant TL software (GE Healthcare).

Carrageenan-induced paw edema in mice

The *in viv*o anti-inflammatory effects of PI3K inhibitor LY294002 and different stilbenoids were studied by measuring carrageenan-induced paw edema in male C57BL/6 mice. The study was carried out in accordance with the legislation for the protection of animals used for scientific purposes (directive 2010/63/EU) and approved by the National Animal Experiment Board (approval number ESAVI/5019/04.10.03/2012, granted September 3, 2012). Paw edema was induced under anaesthesia and all efforts were made to minimize suffering.

Animals were housed under conditions of optimum light, temperature and humidity (12:12 h light:dark cycle, 22±1 °C, 50–60% humidity) with food and water provided ad libitum. One hour prior to carrageenan injection the investigated compounds were delivered i.p. dissolved in a vehicle containing 10% DMSO in PBS (pH 7.4). The mice were anesthetised by intraperitoneal injection of 0.5 mg/kg of medetomide (Domitor® 1 mg/mL, Orion Oyj, Espoo, Finland) and 75 mg/kg of ketamine (Ketalar® 10 mg/mL, Pfizer Oy Animal Health, Helsinki, Finland), and thereafter the mice received a 50-µL intradermal injection in one hindpaw of normal saline containing λ-carrageenan (1.5% w/v). The contralateral paw received 50 µL of saline only and it was used as a control. Edema was measured before and three and six hours after carrageenan injection with a plethysmometer (Ugo Basile, Comerio, Italy).

At six hours the mice were sacrificed and the paw tissue injected with carrageenan and the contralateral tissue injected with the vehicle was collected for analysis into a buffer containing Tris (50 mM, pH 7.4), NaCl (150 mM), and protease and phosphatase inhibitors

phenylmethylsulfonyl fluoride (0.5 mM), sodium orthovanadate (2 mM), leupeptin (0.10 µg/mL), aprotinin (0.25 µg/mL) and NaF (1.25 mM). The tissue was minced and centrifuged (10 min, 10 000g) and the supernatant was collected for ELISA measurements.

Statistics

Results are expressed as mean+standard error of mean (S.E.M.). Statistical significance of the results was calculated by one-way ANOVA with Dunnett's or Bonferroni's post-test. Differences were considered significant at *$p<0.05$, **$p<0.01$, ***$p<0.001$.

AUTHOR INFORMATION

Corresponding Author

*E-mail: eeva.moilanen@uta.fi

ACKNOWLEDGEMENTS

This study was funded by the Finnish Bioeconomy Cluster (FIBIC Ltd), the Finnish Funding Agency for Technology and Innovation (TEKES) and the Medical Research Fund of Tampere University Hospital. We wish to thank Meiju Kukkonen, Salla Hietakangas and Terhi Salonen for excellent technical assistance and Heli Määttä for skillful secretarial help.

REFERENCES

(1) Jeandet, P.; Delaunois, B.; Conreux, A.; Donnez, D.; Nuzzo, V.; Cordelier, S.; Clement, C.; Courot, E. Biofactors. 2010, 36, 331-341.

(2) Chiron, H.; Drouet, A.; Lieutier, F.; Payer, HD.; Ernst, D.; Sanderman H. Plant Physiol.. 2000, 124, 865-872.

(3) Keylor, MH.; Matsuura, BS; Stephenson, CRJ. Chem. Rev. 2015, 115, 8976-9027.

(4) Catalgol, B.; Batirel, S.; Taga, Y.; Ozer, N. K. Pharmacol. 2012, 3, 141.

(5) Patel, K. R.; Scott, E.; Brown, V. A.; Gescher, A. J.; Steward, W. P.; Brown, K. Ann. N. Y. Acad. Sci. 2011, 1215, 161-169.

(6) Piotrowska, H.; Kucinska, M.; Murias, M. Mutat. Res. 2012, 750, 60-82.

(7) McCormack, D.; McFadden, D. J. Surg. Res. 2012, 173, e53-61.

(8) Misiti, F.; Sampaolese, B.; Mezzogori, D.; Orsini, F.; Pezzotti, M.; Giardina, B.; Clementi, M. E. Brain Res. Bull. 2006, 71, 29-36.

(9) Matsui, Y.; Sugiyama, K.; Kamei, M.; Takahashi, T., Suzuki, T.; Katagata, Y.;Ito, Y. J. Agric. Food Chem. 2010, 58, 11112–11118.

(10) Rimando, A. M.; Kalt, W.; Magee, J. B.; Dewey, J.; Ballington, J. R. J. Agric. Food Chem. 2004, 52, 4713-4719.

(11) Holmbom,B.; Willfoer,S.; Hemming,J.; Pietarinen,S.; Nisula,L.; Eklund,P.; Sjoeholm,R. Materials, Chemicals, and Energy from Forest Biomass. 2007, 22, 350–362.

(12) Lee, S. K.; Lee, H. J.; Min, H. Y.; Park, E. J.; Lee, K. M.; Ahn, Y. H.; Cho, Y. J.; Pyee, J. H. Fitoterapia. 2005, 76, 258-260.

(13) Roupe, K. A.; Remsberg, C. M.; Yanez, J. A.; Davies, N. M. Curr. Clin. Pharmacol. 2006, 1, 81-101.

(14) Ko, Y. J.; Kim, H. H.; Kim, E. J.; Katakura, Y.; Lee, W. S.; Kim, G. S.; Ryu, C. H. Int. J. Mol. Med. 2013, 31, 951-958.

(15) Matsuda, H.; Kageura, T.; Morikawa, T.; Toguchida, I.; Harima, S.; Yoshikawa, M. Bioorg. Med. Chem. Lett. 2000, 10, 323-327.

(16) Cheong, H.; Ryu, S. Y.; Kim, K. M. Planta Med. 1999, 65, 266-268.

(17) Hawkins, P. T.; Stephens, L. R. Biochim. Biophys. Acta. 2015, 1851, 882-897.

(18) Fruman, D. A.; Rommel, C. Nat. Rev. Drug Discov. 2014, 13, 140-156.

(19) Yang, Z. Z.; Tschopp, O.; Baudry, A.; Dummler, B.; Hynx, D.; Hemmings, B. A. Biochem. Soc. Trans. 2004, 32, 350-354.

(20) Hers, I.; Vincent, E. E.; Tavare, J. M. Cell. Signal. 2011, 23, 1515-1527.

(21) Di Lorenzo, A.; Fernandez-Hernando, C.; Cirino, G.; Sessa, W. C. Proc. Natl. Acad. Sci. U. S. A. 2009, 106, 14552-14557.

(22) Rommel, C.; Camps, M.; Ji, H. Nat. Rev. Immunol. 2007, 7, 191-201.

(23) Khan, S.; Shehzad, O.; Jin, H. G.; Woo, E. R.; Kang, S. S.; Baek, S. W.; Kim, J.; Kim, Y. S. J. Nat. Prod. 2012, 75, 67-71.

(24) Erasalo, H.; Laavola, M.; Hamalainen, M.; Leppanen, T.; Nieminen, R.; Moilanen, E. Basic Clin. Pharmacol. Toxicol. 2015, 116, 53-61.

(25) Ali, M. A.; Kondo, K.; Tsuda, Y. Synthesis and Nematocidal Activity of Hydroxystilbenes. Chem. Pharm. Bull. 1992, 40, 1130-1136.

(26) Ferrieres, J. Heart. 2004, 90, 107-111.

(27) Park, E. J.; Pezzuto, J. M. Biochim. Biophys. Acta. 2015, 1852, 1071-1113.

(28) Vanhaesebroeck, B.; Guillermet-Guibert, J.; Graupera, M.; Bilanges, B. Nat. Rev. Mol. Cell Biol. 2010, 11, 329-341.

(29) Minihane, A. M.; Vinoy, S.; Russell, W. R.; Baka, A.; Roche, H. M.; Tuohy, K. M.; Teeling, J. L.; Blaak, E. E.; Fenech, M.; Vauzour, D.; McArdle, H. J.; Kremer, B. H.; Sterkman, L.; Vafeiadou, K.; Benedetti, M. M.; Williams, C. M.; Calder, P. C. Br. J. Nutr. 2015, 114, 999-1012.

(30) Fain, J. N. Vitam. Horm. 2006, 74, 443-477.

(31) Calder, P. C.; Ahluwalia, N.; Brouns, F.; Buetler, T.; Clement, K.; Cunningham, K.; Esposito, K.; Jonsson, L. S.; Kolb, H.; Lansink, M.; Marcos, A.; Margioris, A.; Matusheski, N.; Nordmann, H.; O'Brien, J.; Pugliese, G.; Rizkalla, S.; Schalkwijk, C.; Tuomilehto, J.; Warnberg, J.; Watzl, B.; Winklhofer-Roob, B. M. Br. J. Nutr. 2011, 106 Suppl 3, S5-78.

(32) Wymann, M. P.; Solinas, G. Ann. N. Y. Acad. Sci. 2013, 1280, 44-47.

(33) Vlahos, C. J.; Matter, W. F.; Hui, K. Y.; Brown, R. F.. J. Biol. Chem. 1994, 269, 5241-5248.

(34) Kim, J. S.; Kang, C. G.; Kim, S. H.; Lee, E. O. J. Nat. Prod. 2014, 77, 1135-1139.

(35) Pan, M. H.; Chiou, Y. S.; Chen, W. J.; Wang, J. M.; Badmaev, V.; Ho, C. T. Carcinogenesis. 2009, 30, 1234-1242.

(36) Aktan, F. Life Sci. 2004, 75, 639-653.

(37) Collart, M. A.; Baeuerle, P.; Vassalli, P. Mol. Cell. Biol. 1990, 10, 1498-1506.

(38) Hershko, D. D.; Robb, B. W.; Luo, G.; Hasselgren, P. O. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2002, 283, R1140-8.

(39) Hildebrand, D. G.; Alexander, E.; Horber, S.; Lehle, S.; Obermayer, K.; Münck, N. A.; Rothfuss, O.; Frick, J. S.; Morimatsu, M.; Schmitz, I.; Roth, J.; Ehrchen, J. M.; Essmann, F.; Schulze-Osthoff, K. J. Immunol. 2013, 190, 4812-4820.

(40) Fulgenzi, A.; Dell'Antonio, G.; Foglieni, C.; Dal Cin, E.; Ticozzi, P.; Franzone, J. S.; Ferrero, M. E. BMC Immunol. 2005, 6, 18.

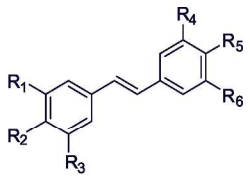
(41) Loram, L. C.; Fuller, A.; Fick, L. G.; Cartmell, T.; Poole, S.; Mitchell, D. J. Pain. 2007, 8, 127-136.

(42) Salvemini, D.; Wang, Z. Q.; Wyatt, P. S.; Bourdon, D. M.; Marino, M. H.; Manning, P. T.; Currie, M. G. Br. J. Pharmacol. 1996, 118, 829-838.

(43) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. Anal. Biochem. 1982, 126, 131-138.

(44) Bradford, M. M. Anal. Biochem. 1976, 72, 248-254.

(45) Leppänen, T.; Korhonen, R.; Laavola, M.; Nieminen, R.; Tuominen, R. K.; Moilanen, E. PLoS One. 2013, 8, e52741.



Compound	R_1	R_2	R_3	R_4	R_5	R_6
Pinosylvin (1)	OH	H	OH	H	H	H
Monomethyl pinosylvin (2)	OMe	H	OH	H	H	H
Resveratrol (3)	OH	H	OH	H	OH	H
Pterostilbene (4)	OMe	H	OMe	H	OH	H
Piceatannol (5)	OH	H	OH	OH	OH	H
Rhapontigenin (6)	OH	OMe	H	OH	H	OH
Astringin (7)	O-β-D-Glc	H	OH	OH	OH	H
Rhaponticin (8)	OH	OMe	H	O-β-D-Glc	H	OH
Pinosylvin monoethyl ether (9)	OEt	H	OH	H	H	H
Pinosylvin monopropyl ether (10)	OPr-*n*	H	OH	H	H	H
Pinosylvin monoallyl ether (11)	OAll	H	OH	H	H	H
Pinosylvin monoisobutyl ether (12)	OBu-*i*	H	OH	H	H	H
Pinosylvin monobenzyl ether (13)	OBn	H	OH	H	H	H

Figure 1: The molecular structures of the studied natural and semi-synthetic stilbenoids.

170x236mm (300 x 300 DPI)

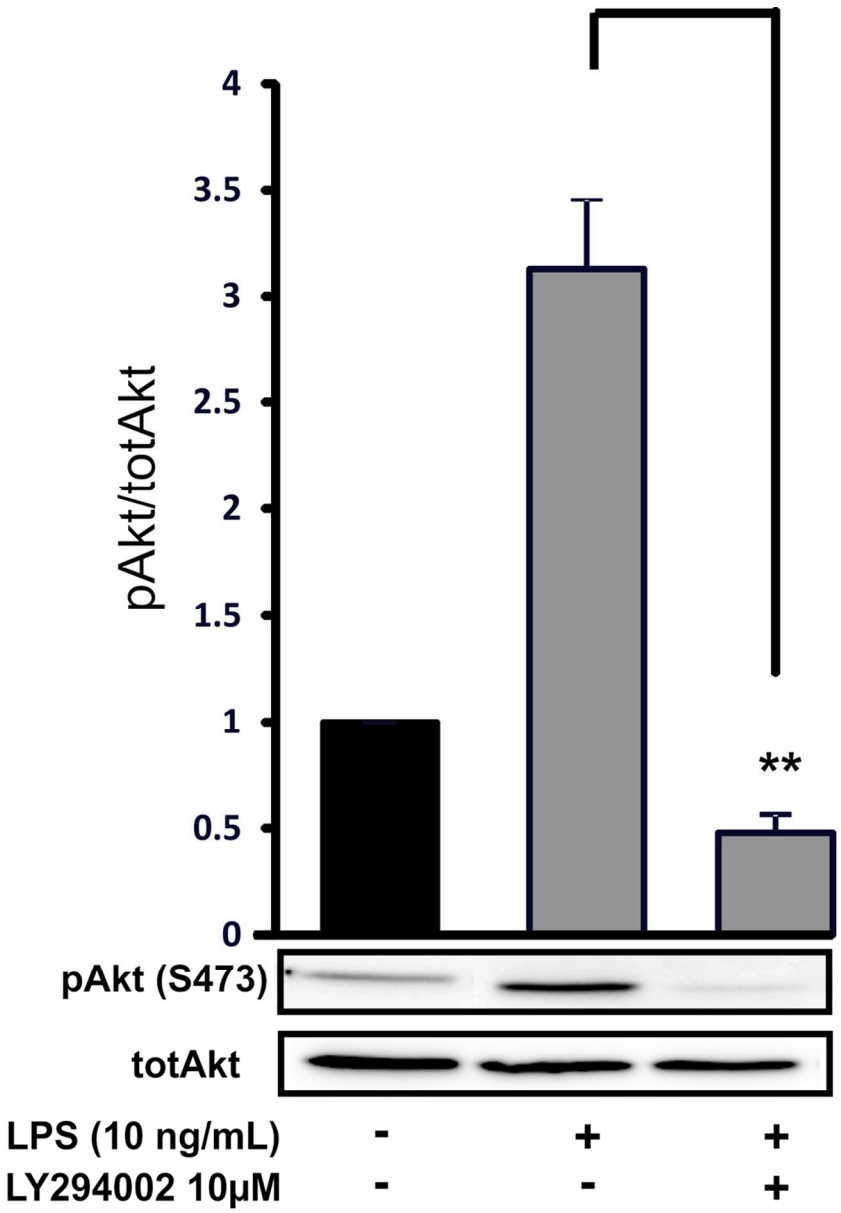


Figure 2: LPS-stimulated phosphorylation of Akt in activated macrophages. J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) in the absence or in the presence of 10 μM LY294002 for 4 hours. Thereafter cellular proteins were extracted and the level of Akt phosphorylation was measured by Western blotting with an antibody specific towards phosphorylated S473 residue. Values are mean + S.E.M., n = 4, **p < 0.01.

89x129mm (300 x 300 DPI)

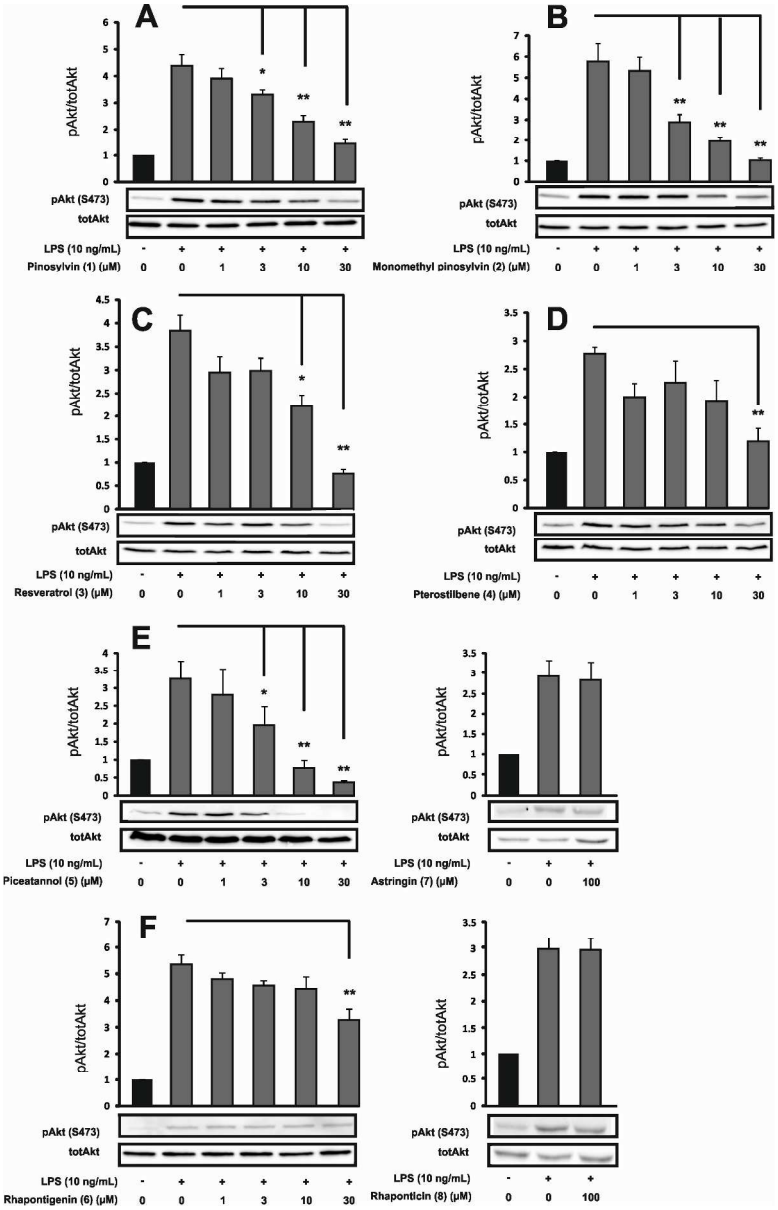


Figure 3: The concentration-dependent effect of natural stilbenoids and stilbenoid glycosides on Akt phosphorylation in activated macrophages. J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) for 4 hours in the presence of increasing concentrations of (A) pinosylvin (1), (B) monomethyl pinosylvin (2), (C) resveratrol (3), (D) pterostilbene (4), (E) piceatannol (5) and astringin (7), and (F) rhapontigenin (6) and rhaponticin (8). Thereafter cellular proteins were extracted and the level of Akt phosphorylation was investigated by western blotting with an antibody specific towards phosphorylated S473 residue. Values are mean + S.E.M., n = 4-6, *p < 0.05 and **p < 0.01.

207x322mm (300 x 300 DPI)

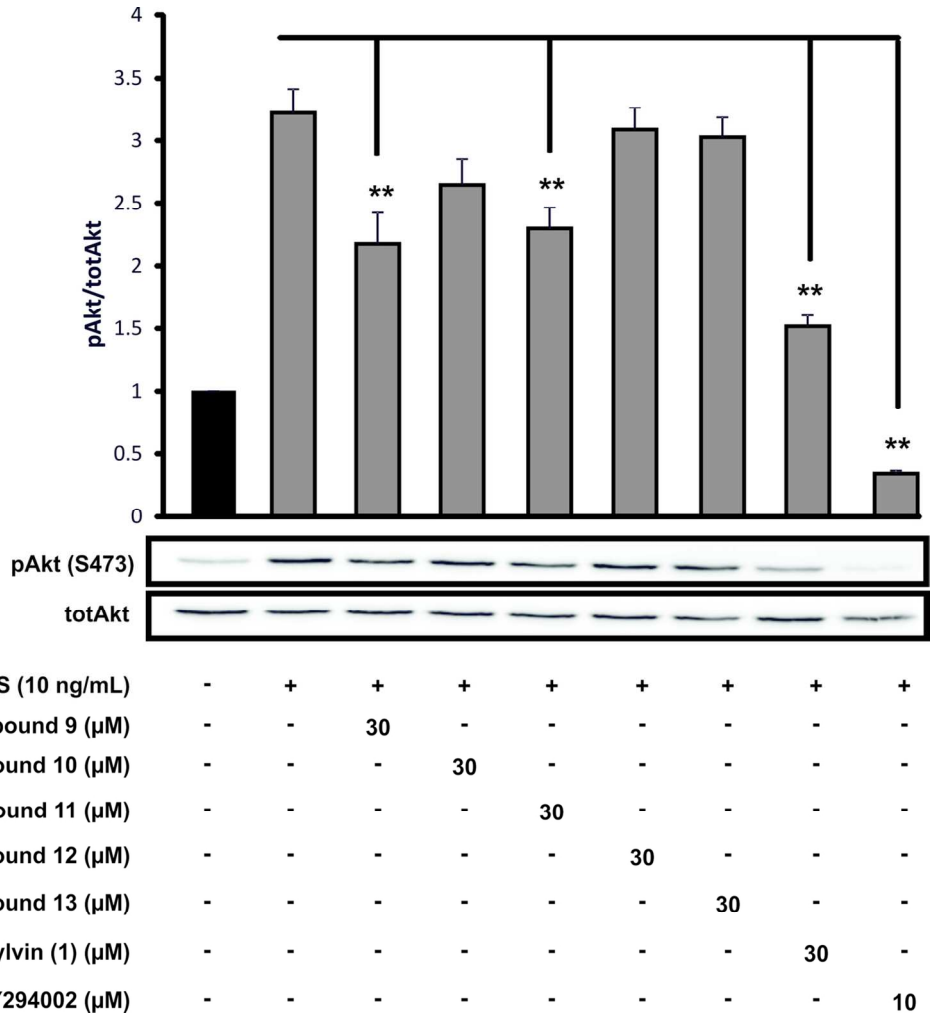


Figure 4. The effect of semi-synthetic pinosylvin derivatives on Akt phosphorylation in activated macrophages. J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) for 4 hours in the presence of the semi-synthetic stilbenoids 9-13 (30 μM). Pinosylvin and LY294002 were used as reference compounds. After incubation cellular proteins were extracted and Akt phosphorylation was investigated by Western blotting with an antibody specific towards phosphorylated S473 residue. Values are mean + S.E.M., n = 4, **p < 0.01.

126x126mm (300 x 300 DPI)

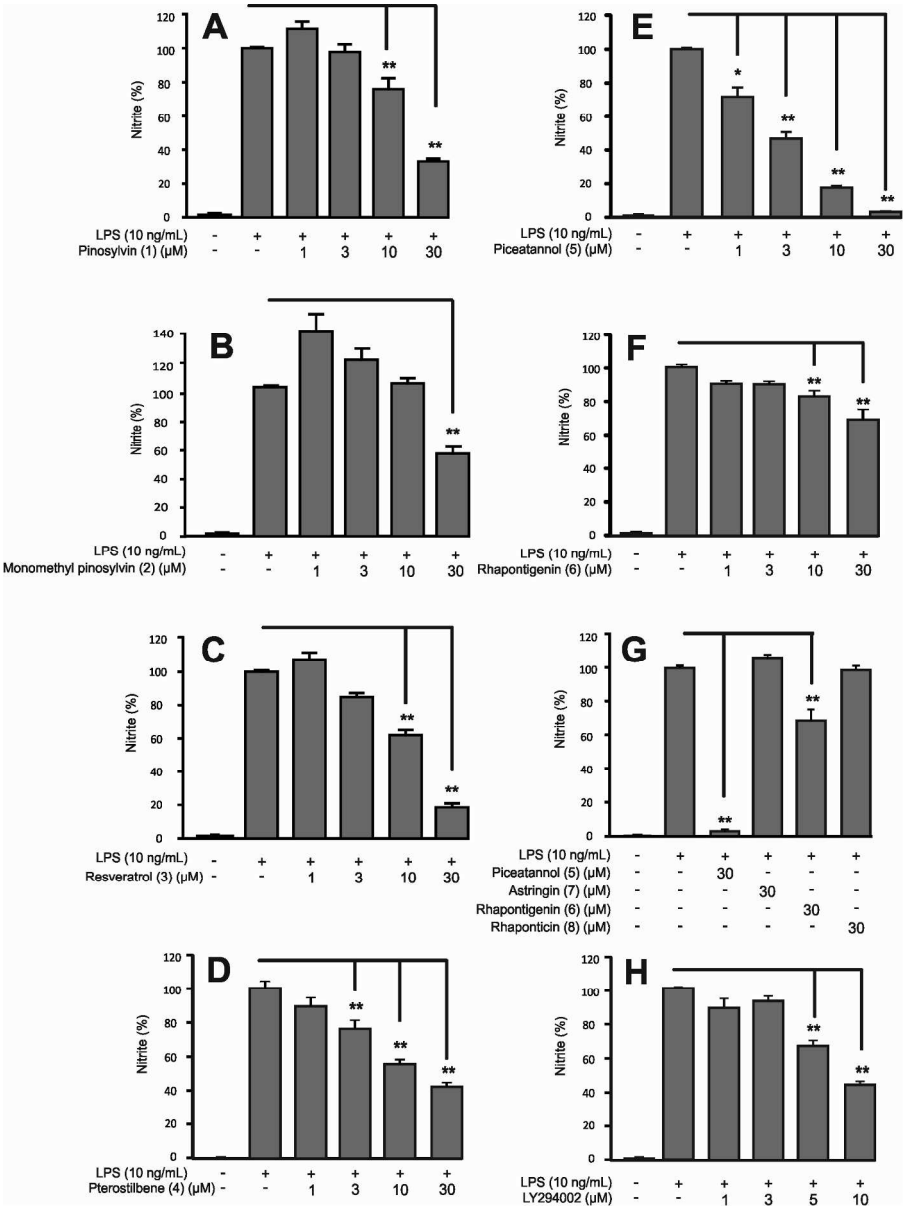


Figure 5: The effects of natural stilbenoids on NO production in activated macrophages. J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) in the presence of increasing concentrations of (A) pinosylvin (1), (B) monomethyl pinosylvin (2), (C) resveratrol (3), (D) pterostilbene (4), (E) piceatannol (5) and (F) rhapontigenin (6) for 24 hours. After incubation the cell culture media were collected. NO production was investigated by measuring the accumulation of its stabile metabolite nitrite in the cell culture media with the Griess method. In addition, the effects of stilbenoid glycosides astringin (7) and rhaponticin (8) on NO production were investigated and compared to their aglycones (G). LY29004 was used as a reference compound (H). Values are mean + S.E.M., n = 4, *p < 0.05 and **p < 0.01.

186x249mm (300 x 300 DPI)

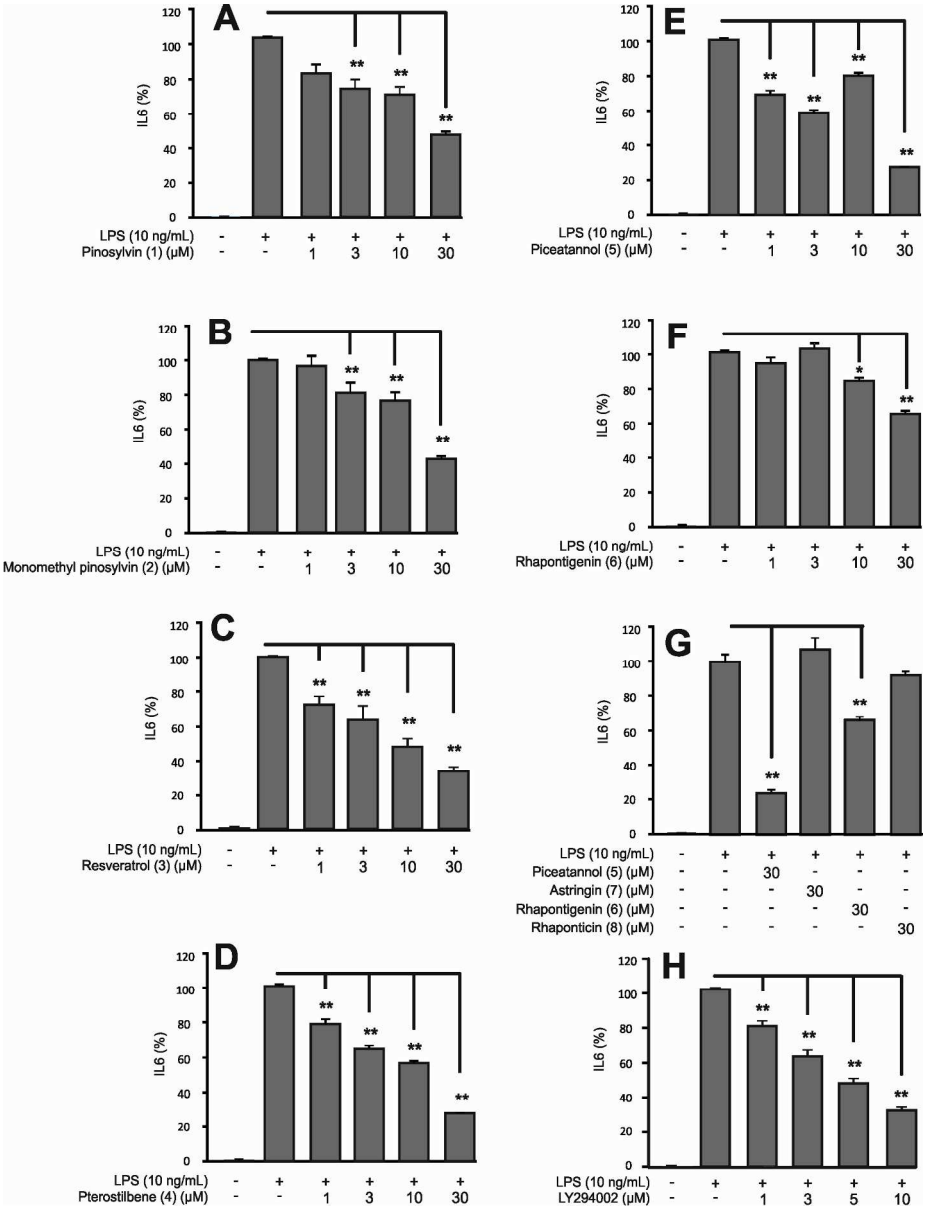


Figure 6: The effects of natural stilbenoids on IL6 expression in activated macrophages. J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) in the presence of increasing concentrations of (A) pinosylvin (1), (B) monomethyl pinosylvin (2), (C) resveratrol (3), (D) pterostilbene (4), (E) piceatannol (5) and (F) rhapontigenin (6) for 24 hours. After incubation the cell culture media were collected and IL6 concentrations were measured with ELISA. In addition, the effects of stilbenoid glycosides astringin (7) and rhaponticin (8) on IL6 production were measured investigated and compared to their aglycones (G). LY29004 was used as a reference compound (H). Values are mean + S.E.M., n = 4, *p < 0.05 and **p < 0.01.

198x258mm (300 x 300 DPI)

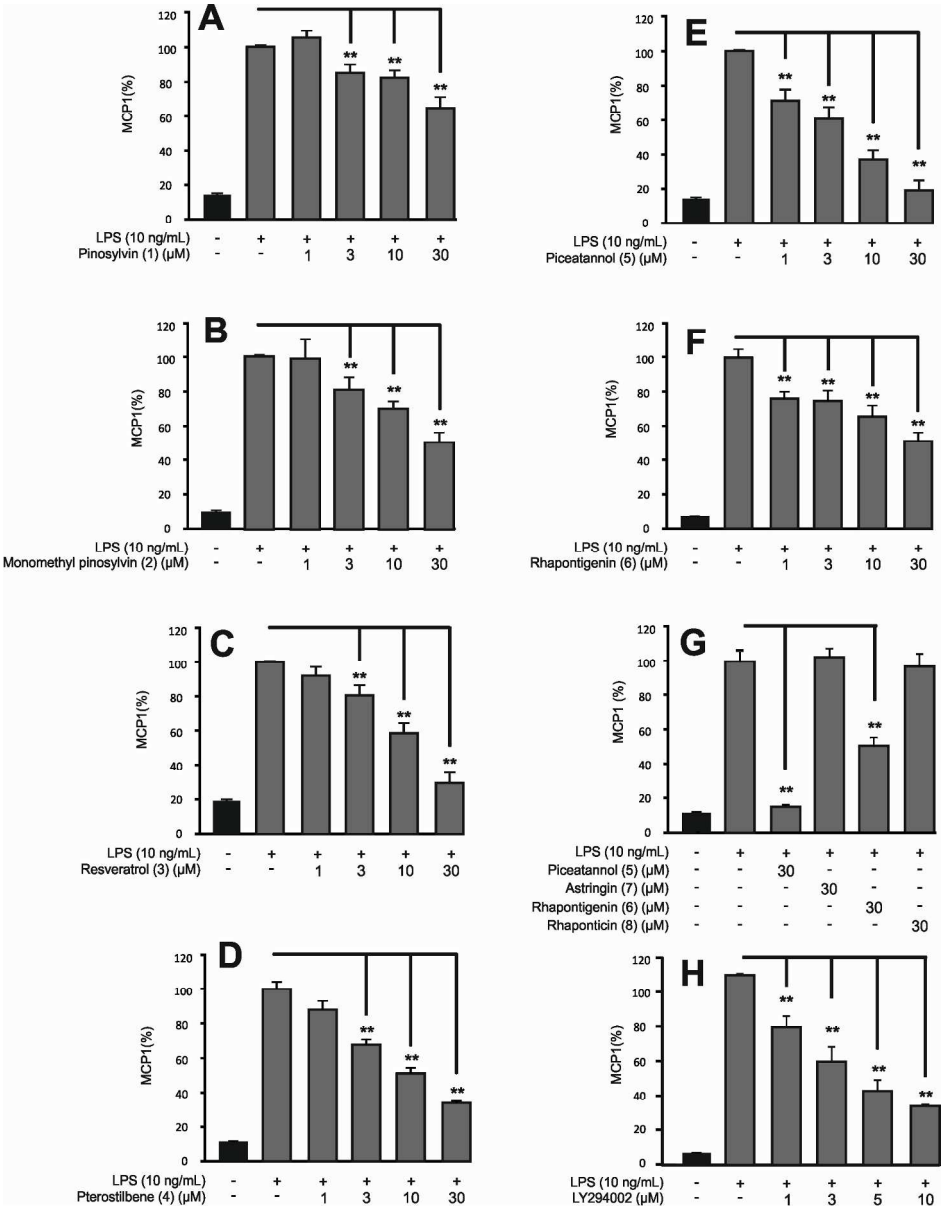


Figure 7: The effects of natural stilbenoids on MCP1 expression in activated macrophages J774 macrophages were stimulated with bacterial lipopolysaccharide (LPS, 10 ng/mL) in the presence of increasing concentrations of (A) pinosylvin (1), (B) monomethyl pinosylvin (2), (C) resveratrol (3), (D) pterostilbene (4), (E) piceatannol (5) and (F) rhapontigenin (6) for 24 hours. After incubation the cell culture media were collected and the concentrations of MCP1 were measured with ELISA. In addition, the effects of stilbenoid glycosides astringin (7) and rhaponticin (8) on MCP1 production were measured and compared to their aglycones (G). LY29004 was used as a reference compound (H). Values are mean + S.E.M., n = 4, **p < 0.01.

201x258mm (300 x 300 DPI)

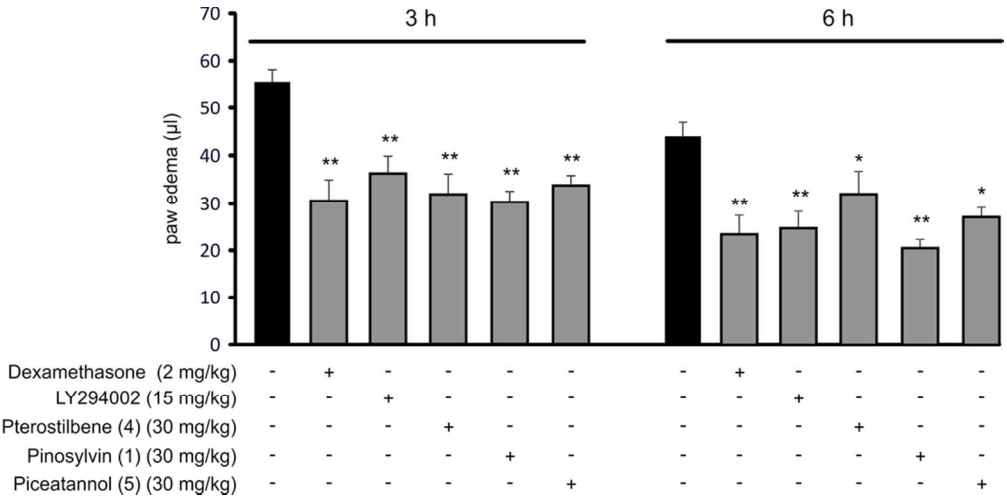


Figure 8: The effects of natural stilbenoids in the carrageenan-induced paw inflammation model in the mouse. The investigated stilbenoids pterostilbene (4), pinosylvin (1) and piceatannol (5) (all 30 mg/kg) or the PI3K inhibitor LY294002 (15 mg/kg) or dexamethasone (2 mg/kg) were administered to the animals intraperitoneally 2 hours prior to carrageenan (1.5% in saline) was injected into the paw, and saline was injected into the contralateral paw to serve as a control. Paw edema was measured before, and 3 and 6 hours after carrageenan injection. Edema is expressed as the difference between the volume changes of the carrageenan-treated paw and the control paw (injected with saline). Values are mean + S.E.M., n = 6 (stilbenoids) or 7 (dexamethasone and LY294002), *p < 0.05 and **p < 0.01.

90x44mm (300 x 300 DPI)

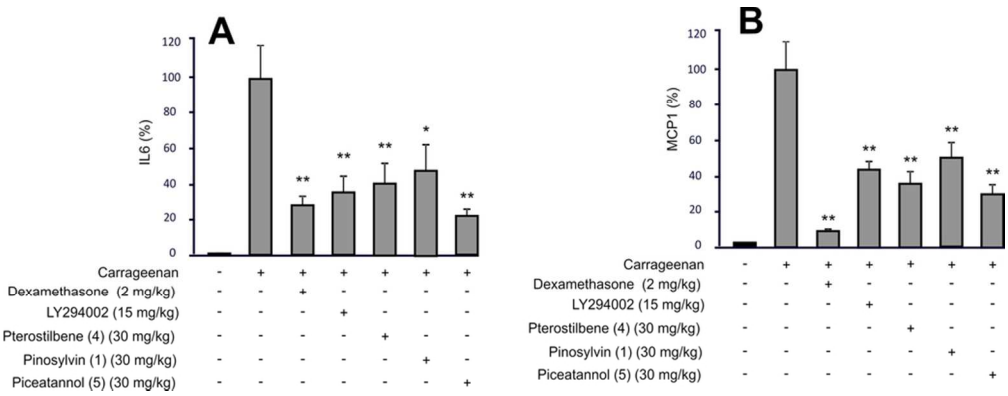


Figure 9: The effects of natural stilbenoids on the expression of the inflammatory cytokines IL6 and MCP1 in the carrageenan-induced paw inflammation model in the mouse. The investigated stilbenoids pterostilbene (4), pinosylvin (1) and piceatannol (5) (all 30 mg/kg) or the PI3K inhibitor LY294002 (15 mg/kg) or dexamethasone (2 mg/kg) were administered to the animals intraperitoneally 2 hours prior to carrageenan (1.5% in saline) was injected into the paw, and saline was injected into the contralateral paw to serve as a control. Six hours after carrageenan exposure the animals were sacrificed and the paw tissue was extracted. The expression of IL6 and MCP1 in the tissue was measured with ELISA. Values are mean + S.E.M., n = 6 (stilbenoids) or 7 (dexamethasone and LY294002), *p < 0.05 and **p < 0.01.

87x33mm (300 x 300 DPI)